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### Peripheral Blood of Koi Fish (*Cyprinus carpio*) Infested by *Argulus japonicus* in Mungkid and Muntilan District, Magelang, Central Java

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#### Abstract

Koi fish (*Cyprinus carpio*) is one of the ornamental fish that has high economic potential. However, *Argulus japonicus* is one of the problems that affect koi fish. It may even causes death in Koi fish. The study was carried out in Mungkid and Mungkilan districts were the biggest koi production in Central Java, Indonesia. This study showed that the neutrophils, monocytes, eosinophils and basophils of infected fish would decreases, except the lymphocytes.

**Key words**: Peripheral Blood, koi fish, *Argulus japonicus*, infestation.

Koi fish (*Cyprinus carpio*) is one of the ornamental fish that has high economic potential. The average production of koi fish reaches 72,000 tons per year at a price of Rp. 100.000-200.000 for domestic market and Rp. 1.000.000-25.000.000 for the international market (Sunarto, 2005). However, *Argulus japonicus* is one of the problem that affect koi fish. The prevalence of *Argulus japonicus* was recorded at 4.16% (Wahyuni *et al.*, 2013).

*Argulus* attacks on the fins, skin, gills, and the entire surface of the host's body (Walker, 2008). The wounds inflicted by the Argulus will cause bleeding and damage that may result in inflammation, followed by other tissue damage (Notash, 2012). It will affect the growth, production, and even death (Alifudin *et al.*, 2002).

Through this study, on the peripheral blood and histopathology of *Argulus japonicus*, in the district of Mungkid and Muntilan, Magelang, Central Java was done to create awareness among the fish farmers (Badan Pusat Statistik, 2013).

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#### Materials and Methods

The research was conducted from 29 February to 7 March 2016. The positively infected koi fishes with size 5-15 cm were collected from two districts, Mungkid (14) and Muntilan (29). The analysis was conducted at the Laboratory of Balai Benih Ikan Ngrajek, Magelang; Laboratory of Fisheries and Marine Faculty-Universitas Airlangga, Surabaya and Laboratory of Pathology Faculty of Veterinary-Universitas Airlangga, Surabaya. This study also added positive control of healthy koi fishes from each place to compare them with infected samples.

All parts of the skin and fins were scrapped carefully for Parasitological examination, and were examined under a microscope (Ebrahimi *et al.*, 2018). Parasite identification was performed by using article of diagnostic keys (Walker *et al.*, 2004). The prevalence level was calculated by using prevalence formula (Kabata, 1985).

The blood was drawn by 1 ml syringe coated with EDTA (Ethylene Diamine Tetra Acetic) 10% (Syahida *et al.* 2013). The dried blood smear preparations were fixed in methanol solution for 5-10 minutes. Immersed in a 10% Giemsa solution for 10-15 minutes after dry. Rinsed with aquadest and dried. The slides were observed under a microscope to record the changes in the leukocytes in the different locations under study (Svobodova and Vykusova, 1991).

#### **Results and Discussion**

*Argulus* was found in the mandibles, labial spines and stylets of the mouth, in the fin and skin of koi fish (*Cyprinus carpio*) (Walker *et al.*, 2004) Fig 1.



Fig 1. Koi fish infected with Argulus japonicus

The prevalence level of koi fish infested by Argulus in Mungkid and Muntilan Districts are presented in Table I.

The leukocytes examined in this study consisted of lymphocytes, monocytes, neutrophils, eosinophils and basophils (Fig 2). Leukocytes were examined under 1000x magnification.

The leukocytic distribution in the healthy, medium level of infected koi fish is presented in Table II. The results showed that the average percentage of lymphocytes from normal koi fish in Mungkid and Muntilan was 89.5% and 89.6%. (Svobodová & Vykusová 1991) recorded that lymphocytes in goldfish ranged from 76 to 97.5%. The average percentage of infected koi lymphocytes in Mungkid and Muntilan decreased according to the severity of  $\ensuremath{\textbf{Table I}}$  . The prevalence level of koi fish in Mungkid and Muntilan

No. of complex texted	Region			
No. of samples tested	Mungkid	Muntilan		
Total	13	29		
Positive	4	16		
Prevalence (%)	30.77	55.17		

#### infestation of Argulus.

The decrease in the number of lymphocytes is due to the infection with *Argulus japonica* and the concomitant decrease in the lymphocytes since they are involved in the immune response (Voigt and Swist, 2011).

The study showed that the average percentage of neutrophils from normal koi fish in Mungkid and Muntilan were 4.8% and 4.6%. The average percentage of neutrophils from infected koi fish of low category in Mungkid and Muntilan increased were 5.7% and 5.4% respectively. The average percentage of infected koi neutrophils of medium category infection in Mungkid and Muntilan were 8.25% and 8.5% respectively. According to Harikrishnan *et al.*, (2010), the increase in neutrophils acts as the first line of defense. The main function of neutrophils was

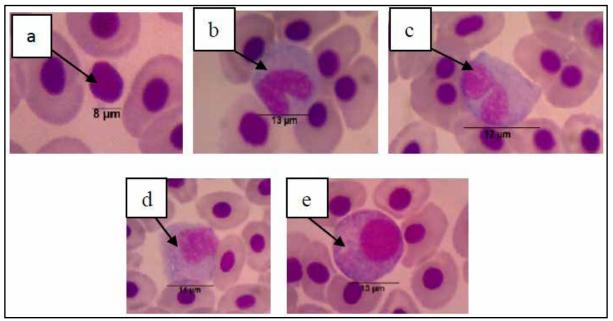


Fig 2. Leukocytes examination a. Lymphocytes; b. Monocytes; c. Neutrophils; d. Eosinophils; e. Basophils

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Location	Argulus japonicus parasite	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
	Normal (positive control)	89.5	4.8	4	1.4	0.14
Mungkid	few (1-5-5 Argulus)	84.5	5.7	5	4.2	0.5
	medium (6-10 Argulus)	79.7	8.25	7	4.5	0.5
Muntilan	Normal (control)	89.6	4.6	3.9	1.5	0.2
	few (1-5 Argulus)	85.1	5.4	4.9	4.1	0.35
	medium (6-10 Argulus)	78	8.5	6.5	6.5	0.5

Table II. The leucocyte distribution in normal, low and medium level of infected koi fishes in Mungkid and Muntilan (%)

the destruction of foreign matter through the phagocytic process by chemotaxis and destruction by lysosomal enzymes in phagolysosomes.

The study showed that the average monocyte percentage of normal koi fish in Mungkid was 4%, while in Muntilan was 3.9%. The average percentage of monocytes in low level of ion infect koi fish in Mungkid and Muntilan were 5% and 4.9%, respectively. The medium category of infection in Mungkid and Muntilam showed 7% and 6.5% monocytes in the two regions. The inflammatory process during tissue damage by infection or antigen-antibody reaction. which would increase monocyte production to two times more (Maftuch, 2007). The main function of monocytes was phagocytosis. Monocytes swallow and destroy organisms which could not be controlled by neutrophils, especially fungi. Increased monocytes showed chronic infections or inflammatory responses, in addition, the increased monocytes might also indicate an acute-phase of recovery (Voigt and Swist, loc. cit).

The eosinophil percentage from normal koi fish in Mungkid was 1.4%, while in Muntilan it was 1.5%. The percentage of eosinophils in normal fish blood ranged from 0.78-2.00% (Affandi and Tang, 2002). The percentage of infected eosinophils of infected koi fish low category in Mungkid and Muntilan were between 4.2% and 4.1%. In the medium category of infected fish in Mungkid and Muntilan were 4.5 and 6.5%. The eosinophils were a type of leukocytes associated with parasitic infections, thus signifying the presence of parasites (Robert, 1989).

The study showed that the average of basophil percentage of normal koi fish

in Mungkid was 0.14%, while Muntilan sub-districts was 0.2%. The average percentage of basophil from infected koi fish category, low in Mungkid and Muntilan were 0.5% and 0.35. The average percentage of basophil infected koi fish in Mungkid and Muntilan was 0.5%. Basophil percentages in normal koi fish ranged from 0 to 0.5% (Svobodová & Vykusová, *loc. cit*). Basophil images were rarely found with Giemsa staining and whose function is not known clearly (Bijanti, 2005) Table II.

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## *In vitro* Evaluation of Acaricidal Property of *Acalypha Indica* (Kuppaimeni) against Ticks Infesting Sheep

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#### Abstract

Laboratory tests were carried out to determine the toxicity of the ethanolic extract of Acalypha indica on engorged females and larvae of sheep tick Haemaphysalis intermedia using the Adult Immersion Test (AIT) and the Larval Packet Test (LPT). It was observed that the ethanolic extract of A. indica at 30% concentration showed acaricidal activity. In AIT, egg laying was lower in ticks exposed to different concentrations of A. indica compared with those exposed to 30% ethanol used as control. In LPT, there was increasing mortality of tick larvae with increasing concentrations of A. indica as opposed to no mortality in the control.

**Key words:** Acalypha indica, Acaricide and Haemaphysalis intermedia.

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Haemaphysalis intermedia ticks are considered to be the most prevalent sheep tick species in Tamil Nadu, causing direct damage such as blood loss, hide injuries, irritation and inoculation of toxins and indirect damages by transmission of *Babesia* spp., *Anaplasma* spp. and *Theileria* spp. to the host. Tick control mainly depends on synthetic acaricides but has been complicated by the emergence of drug resistance (Miller et al., 2007). Hence the use of extracts from various plants is being studied because they are potentially less toxic to the animals and are safer for the environment. Phytoacaricides can be developed from many compounds isolated from plants. These can act on ticks by reducing their development, survival and reproductive rate (Bagavan et al., 2009).

The Acalypha indica (Kuppaimeni) is